Claims

1. A bovine beta-casein gene targeting vector comprising (1) a first region having a length of 5 to 12 kb which is homologous to the promoter and its flanking nucleic acid sequences of bovine beta-casein gene, and comprising exon 1, intron 1, and exon 2 of bovine beta-casein gene; (2) a region for cloning a nucleic acid coding for desired proteins; (3) a region for coding a positive selection marker; (4) a second region having a length of 2.8 to 3.5 kb which is homologous to the nucleic acid sequences of bovine beta-casein gene, and comprising exon 5, 6, 7 and 8, and intron 5, 6 and 7 of bovine beta-casein gene; wherein the nucleic acid segment corresponding to the first region is located upstream to the nucleic acid segment corresponding to the second region in the 5′-3′ arrangement of beta-casein gene.

2. The vector according to claim 1, wherein the length of the first region is 5.5 to 10kb.

3. The vector according to claim 1, wherein the length of the second region is 3.0 to 3.2 kb.

4. The vector according to claim 1, wherein the positive selection marker is selected from the group consisting of neomycin (Neo), hygromycin (Hyg), histidinol dehydrogenase gene (hisD) and guanine phosphosribosyltransferase (Gpt).

5. The vector according to claim 1, wherein the vector further comprises a region for a negative selection marker.

6. The vector according to claim 5, wherein the negative selection marker is Diphtheria toxin (DT) gene.

7. A vector according to claim 1 or 5 which is pBCKI Ⅰ, pBCKI Ⅱ, pBCKIDT Ⅰ or pBCKIDT Ⅱ, is presented in FIG. 1, FIG. 2, FIG. 16, or FIG. 3, respectively.

8. A bovine somatic cell which is beta-casein gene-targeted with the vector according to claim 1 or 5.

9. An embryo which is nuclear-transferred with the bovine somatic cell according to claim 8.

10. A method for producing a bovine beta-casein gene-targeted somatic cell which comprises the steps of (1) introducing the bovine beta-casein gene-targeting vector according to claim 1 or 5 into an bovine somatic cell; (2)occurring homologous recombination events in the bovine somatic cell; and (3) selecting the bovine beta-casein gene-targeted somatic cell with a desired gene by homologous recombination.

11. The method according to claim 10, wherein the vector in the step (1) is introduced into cells in form of linearized or deleted form lacking plasmid vector backbone.

12. A method for generating transgenic cattle which comprise the steps of (1) introducing the bovine beta-casein gene-targeting vector according to claim 1 or 5 into a bovine somatic cell; (2) occurring homologous recombination events in the bovine somatic cell; (3) selecting the bovine beta-casein gene-targeted somatic cell with a desired gene by homologous recombination; (4) introducing the gene-targeted cell into a nuclear-removed bovine embryo to produce a nuclear-transferred embryo ; and (5) implanting the embryo into a recipient.

13. A method obtaining a large scale of desired proteins from milk of the transgenic cattle, in accordance with the method of claim 12.